Journal of Biological Control, 29(4): 187-193, 2015

Research Article

Effect of temperature on the infectivity of entomopathogenic nematodes against shoot borer (*Conogethes punctiferalis* Guen.) infesting ginger (*Zingiber officinale* Rosc.)

RASHID PERVEZ*, SANTHOSH J. EAPEN, S. DEVASAHAYAM and T. K. JACOB
Division of Crop Protection
ICAR-Indian Institute of Spices Research, Kozhikode (Kerala) 673 012 – India
**Corresponding author E-mail:* rashid_pervez@rediffmail.com

ABSTRACT: The attachment, penetration, infectivity and multiplication of eight native isolates of Entomopathogenic nematodes (EPNs), *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Steinernema* sp. (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06), *Oscheius* spp. (IISR-EPN 04, 05, and 08) and *O. gingeri* were tested against larvae of *Conogethes punctiferalis* at different temperatures *viz.,* 20, 25, 30 and 35 °C. The temperature significantly affected attachment, penetration, pathogenicity and multiplication ability of infective juveniles (IJs) of all tested EPNs. Among the test temperatures, maximum mortality of larvae was found at 30 °C followed by 25 °C, whereas the least mortality was recorded at 20 and 35 °C. Maximum number of infective juveniles was multiplied at 30 °C, however minimum multiplication was recorded at 35 °C. Among the test EPNs, no multiplication of *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 03) and *O. gingeri* was recorded at 20 °C. IJs attached to larvae of *C. punctiferalis* in higher number after 6 h at 25 and 30 °C. Whereas, maximum number of IJs penetrated into *C. punctiferalis* larvae at 30 °C. Therefore, the optimal temperature for infection and development for all promising EPNs was 30 °C.

KEY WORDS: Entomopathogenic nematodes, ginger, shoot borer, temperature

(Article chronicle: Received: 04-12-2015; Revised: 13-12-2015; Accepted: 18-12-2015)

INTRODUCTION

One of the major constraints in agricultural production in India is sustained losses due to insect pests, diseases and weeds, but in many instances it has been used to denote insects alone. One or more insect pests always associated with every crop, but not all these pests are of economic injury level, their control is one of the major requirements for increase in crop productivity.

Ginger (*Zingiber officinale* Rosc.) is an important spice and medicinal crop grown in India. However, ginger production has shown a downward trend due to several reasons, among which infestation by diseases and insect pests is a major reason. Among the insect pests, shoot borer (*Conogethes punctiferalis* Guen.) is the most serious, in Kerala which indicated that when 50 percent of the pseudostems in a plant are affected, there was a significant reduction of 38 g of yield per plant (Koya *et al*., 1986). Yield losses of 25 percent have also been reported when 23 to 24 percent of a plant's pseudostems are infested and the pest was reported to cause 40 percent yield loss in Kottayam and Idukki districts in Kerala (Nybe, 2001).

Excessive and indiscriminate use of pesticides for the management of this pest could result in pesticide residues in the produce affecting human health and also causing other ecological hazards. There has been a renewed interest in developing environment- friendly pest management schedules in agriculture. Entomopathogenic nematodes (EPNs) have got little attention by researchers though they have a great potential in reducing pest population and with little manipulation their role can be enhanced (Gaugler and Kaya, 1990; Ali *et al*., 2005).

Temperature is one of the most important factors limiting the success of EPNs (Choo *et al*., 2002; Jian *et al*., 2002; Chen *et al*., 2003). It directly influences host searching (Bilgrami and Gaugler, 2007; Susurluk, 2008), pathogenicity (Aydin, 2005; Pervez *et al.,* 2008) and survival (Kung *et al*., 1991; Ali *et al*., 2007; 2009). EPNs are poikilothermic, temperature plays an important role in their survival, infectivity, time of death, development and reproduction. The level of temperature ranges for survival, infectivity and development vary with the EPN species and strains and such EPNs are able to survive at habitat temperatures that undergo daily and/or seasonal cycles of

fluctuation. For success in biocontrol applications, knowing the distribution of beneficial traits like temperature tolerance among EPN species can be beneficial in selecting the optimum candidate for use in a particular geographic area or even microclimate.

Hence, the present study was carried out to test the attachment, penetration, infectivity and multiplication of eight native EPNs namely, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Steinernema* sp. (IISR-EPN 03), *S. carpocapsae* (Weiser, 1955; Wouts *et al*., 1982), *Oscheius* spp. (IISR-EPN 04, 05, and 08) and *O. gingeri* (Pervez *et al*., 2013) against *C. punctiferalis* larvae (SBL) at different temperatures *viz*., 20, 25, 30 and 35 ˚C.

MATERIALS AND METHODS

Entomopathogenic nematodes sources

Infective juveniles (IJs) of tested EPNs were obtained from nucleus culture of nematodes maintained in the Nematology Laboratory. All tested EPN were cultured as per the procedure described by Kaya and Stock (1997). Fresh harvested IJs were surface sterilised with 0.1% Hyamine solution and stored in distilled water in tissue culture flasks for study.

Insect sources

Greater wax moth, *Galleria mellonella* L. reared on artificial diet as per the procedure described by David and Kurup (1988) and test insect, *C. punctiferalis* were collected from ginger fields of IISR Experimental Farm, Peruvannamuzhi and farmer's fields at Kozhikode and Waynand Districts of Kerala and Kodagu Districts (Karnataka). The larvae were sorted out and those of third instar were taken for the present study.

Effect of temperature on the IJs attachment to *Conogethes punctiferalis*

Attachment of test EPNs to *C. punctiferalis* evaluated in petri plates. One larva of *C. punctiferalis* was kept in each plate and 100 IJs of tested EPNs were released and kept test temperatures. The number of IJs attached to the SBL was counted by washing the insect in a counting dish with distilled water after 3, 6, 12 and 24 h. The treatments were replicated 10 times for each washing interval.

Effect of temperature on the rate of IJs penetration into *Conogethes punctiferalis*

The penetration rate assay was conducted as described by Caroli *et al*. (1996). About 100 IJs of respective EPNs were inoculated in the petri plate containing one *C. punctiferalis*/plate and kept at 20, 25, 30 and 35 ˚C. Each treatment consisted of ten replicates. Number of penetrated IJs was determined by dissecting the dead cadaver in Ringer's solution after 72 h.

Effect of temperature on the infectivity of EPNs

Infectivity of test EPNs against *C. punctiferalis* was tested in petri plates. Ten larva of test insect was kept with pieces of ginger pseudostem in each plate. Five hundred IJs of each test EPNs were inoculated and kept at 20, 25, 30 and 35 ˚C in BOD and the mortality was recorded after 72 h. For each EPNs and temperature combination evaluated separately and replicated 10 times along with control (only water). The mortality was calculated as percentages.

Effect of temperature on the multiplication of EPNs

EPN infected dead *C. punctiferalis* larvae at 30 ˚C were removed from the petri plate and kept on White trap (White, 1927) at test temperature regimes *viz.,* 20, 25, 30 and 35 ˚C for emergence of infective juveniles. IJs were collected daily, till the emergence stopped in about 15 days. From this collection, the total emerged populations of EPNs were counted thrice under a stereoscopic binocular microscope with the help of Syracuse counting dish and mean values were worked out.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range test. Before analysis, data of penetration and multiplication of the nematodes were square root-transformed and those of percentages of insect mortalities were arcsine transformed. All means were transformed back to the original units for presentation.

RESULTS AND DISCUSSION

The result shows that attachment, penetration, per cent mortality of shoot borer larva and multiplication of infective juveniles of all test EPNs were influenced by temperatures.

IJs attachment to *Conogethes punctiferalis*

Attachment of IJs was significantly affected by the temperatures. For all test temperature, IJs attachment was higher at 30 ˚C than at 25 ˚C. Three hours after their inoculation and at all temperatures, IJs were found attached to SBL. However, maximum attachment of IJs was found after 6 and 12 h post exposure (Fig. 1).

IJs penetration into *Conogethes punctiferalis*

All tested EPNs IJs penetrated into *C. punctiferalis*, whereas significant differences (df = 7, 38; F= 7.39; *P* =

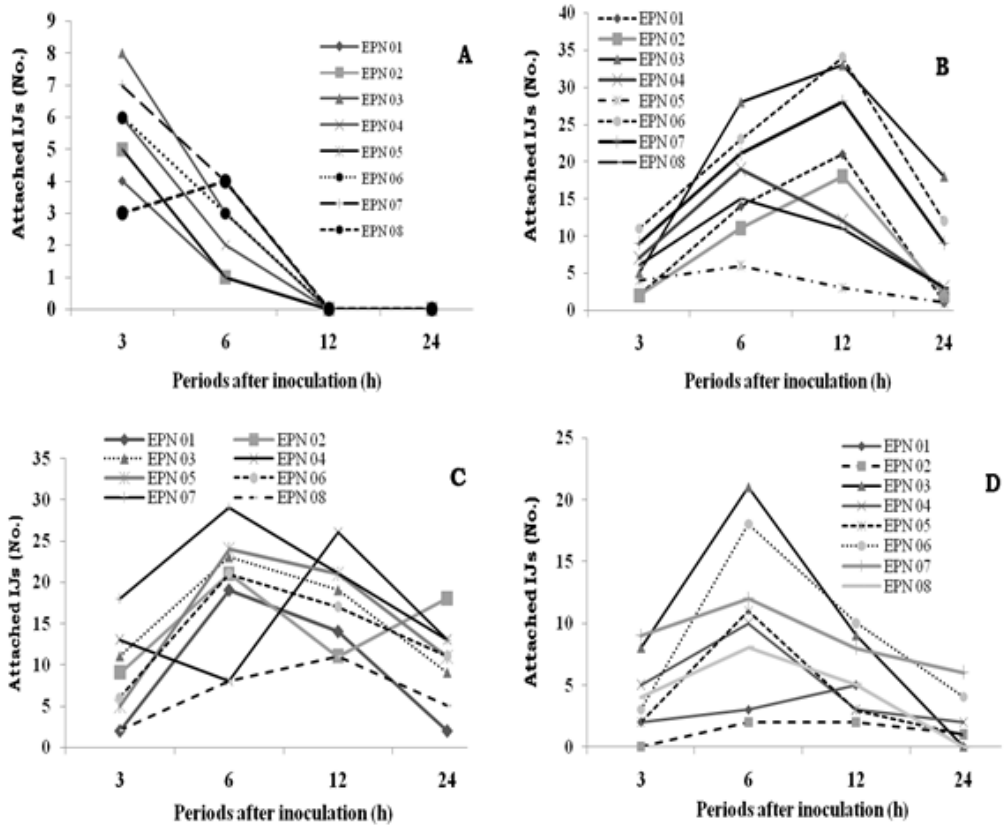

Fig. 1. Influence of different temperature levels on attachment of EPNs against shoot borer larva; A- 20 °C; B - 25 °C; C- 30 °C; D - 35 °C. EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); EPN 02 - *Steinernema* sp. (IISR-EPN 02); EPN 03 - *Steinernema* sp. (IISR-EPN 03); EPN 04 - *Oscheius* sp. (IISR-EPN 04); EPN 05 - *Oscheius* sp. (IISR-EPN 05); EPN 06 - *S. carpocapsae* (IISR- EPN 06); EPN 07 – *O. gingeri* (IISR-EPN 07) and EPN 08 - *Oscheius* sp. (IISR-EPN 08).

0.003) was found in the penetration of IJs. Among the tested temperature, maximum IJs penetration recorded at 30 ˚C followed by 25 ˚C, whereas lesser number at 20 ˚C. Out of the test EPNs and temperature combination, maximum IJs of *Steinernema* sp. (IISR-EPN 03) penetrated into *C. punctiferalis* (14.1 IJs/larva) 30 ˚C, however, the fewest number IJs (0.7 IJs/larva) of *O. gingeri* penetrated into the test insect body at 20 ˚C (Table 1).

Table 1. Number of IJs penetrated in the shoot borer larvae at different temperatures

Entomopathogenic nematodes / Temperatures	Number of IJs/larva			
	20 °C	25 °C	30 °C	35 °C
Heterorhabditis sp. (IISR-EPN 01)	2.7^{c} (0.95)	8.8^{b} (1.32)	11.9^{b} (2.23)	4.9^{abc} (1.66)
Steinernema sp. (IISR-EPN 02)	2.9^{c} (0.99)	7.8^{bc} (1.03)	9.1^{cd} (1.97)	3.9^{cd} (1.45)
Steinernema sp. (IISR-EPN 03)	4.8^{a} (1.14)	11.5^{a} (1.96)	14.1^{a} (2.96)	3.6^{d} (1.43)
Oscheius sp. (IISR-EPN 04)	1.1^{de} (0.88)	3.1^{d} (1.10)	5.2^{f} (1.93)	3.1^{d} (1.29)
Oscheius sp. (IISR-EPN 05)	1.7^{d} (0.95)	2.2^{e} (0.92)	5.8^{ef} (1.14)	4.2^{bcd} (1.32)
S. carpocapsae (IISR-EPN 06)	3.6^{b} (0.84)	8.0^{b} (1.15)	10.3^{bc} (2.21)	5.5^{a} (1.43)
O. gingeri (IISR-EPN 07)	0.7^{e} (0.67)	4.6^{c} (1.90)	7.4^{e} (2.27)	5.2^{ab} (1.03)
Oscheius sp. (IISR-EPN 08)	2.7^{c} (0.67)	2.0^{e} (1.15)	4.2^{f} (1.55)	1.7^{e} (0.95)

Standard deviation are in parenthesis

Infectivity of EPNs against *Conogethes punctiferalis*

All the test EPNs were pathogenic against SBL at test temperatures but the rate of mortality was vary. At all temperatures studied *Steinernema* sp. (IISR-EPN 03) showed highest virulence and *Oscheius* sp. (IISR-EPN 08) the lowest one. No mortality of *C. punctiferalis* was recorded in the controls. Among the test temperatures, maximum mortality of insect was found at 30 ˚C followed by 25 ˚C, whereas the least mortality of *C. punctiferalis* was recorded at 20 and 35 ˚C. Out of the test EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 03) and *O. gingeri* was found more pathogenic, its brought about cent per cent mortality of *C. punctiferalis* at 30 ˚C, whereas *S. carpocapsae* (IISR-EPN 06) caused 100% mortality at 25 ˚C (Fig. 2).

Multiplication of EPNs

Temperature affect the production of IJs and the level of multiplication significantly varied (df = 7, 18; F=36.41; $P < 0.0001$) within EPN isolates and temperature regimes. Among the test temperatures, maximum number of IJs was obtained at 30 ˚C followed by 25 ˚C, while minimum multiplication was recorded at 35 ˚C. Among the test EPNs, highest number (1,16,917 IJs/larva) of infective juveniles of *O. gingeri*, followed by *Oscheius* sp. (IISR-EPN 04) (1,04,771 IJs/larva), whereas, minimum number of IJs was recorded of *S. carpocapsae* (IISR-EPN 06)) (41, 569 IJs/larva) multiplied at 30 ˚C. Out of the test EPN isolates, no multiplication of *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 03) and *O. gingeri* was recorded at 20 ˚C (Fig. 3).

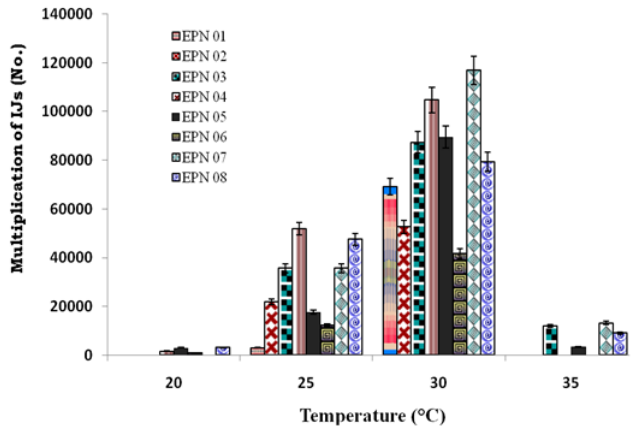

Fig. 3. Influence of different temperature levels on multiplication of EPNs. EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); EPN 02 - *Steinernema* sp. (IISR-EPN 02); EPN 03 - *Steinernema* sp. (IISR-EPN 03)); EPN 04 - *Oscheius* sp. (IISR-EPN 04); EPN 05 - *Oscheius* sp. (IISR-EPN 05); EPN 06 - *S. carpocapsae* (IISR- EPN 06); EPN 07 – *O. gingeri* (IISR-EPN 07) and EPN 08 - *Oscheius* sp. (IISR-EPN 08).

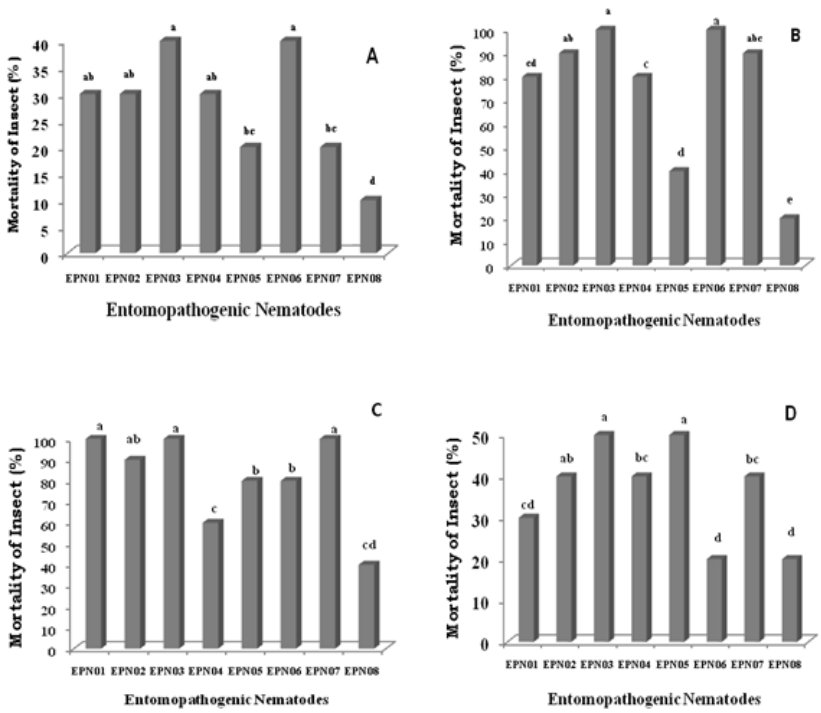

Fig. 2. Influence of different temperature levels on infectivity of EPNs against shoot borer larva. A- 20 °C; B - 25 °C; C- 30 °C; D - 35 °C. EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); EPN 02 - *Steinernema* sp. (IISR-EPN 02); EPN 03 - *Steinernema* sp. (IISR-EPN 03); EPN 04 - *Oscheius* sp. (IISR-EPN 04); EPN 05 - *Oscheius* sp. (IISR-EPN 05); EPN 06 - *S. carpocapsae* (IISR- EPN 06); EPN 07 – *O. gingeri* (IISR-EPN 07) and EPN 08 - *Oscheius* sp. (IISR-EPN 08).

Exposure to extremes of temperature is damaging for nematodes but the extent and nature of damage depends on the duration of exposure. Obviously, the success of a biocontrol programme using EPNs will largely depend on the performance of nematodes at these temperatures.

Pathogenicity studies have shown considerable inter and intraspecific variations in infectivity of different isolates of entomopathogenic nematodes (Karunakar *et al*., 1992; Menti *et al*., 2000; Pervez *et al*., 2012) which have been attributed to the variation in the ability of the IJ to find and enter a host (Griffin *et al.*, 1993; Sankaranarayanan *et al*., 2011) as well as the different host susceptibility among various insects (Ali *et al*., 2008; Pervez *et al*., 2012) or insect stages (Kaya and Hara, 1981; Premchandra *et al*., 2007; Pervez, 2010). However, there is considerable variation in the infectivity of EPNs and no single species or strain is suitable for controlling all or even most insect species (Simoes and Rosa, 1996).

Effect of temperatures on nematode performance varies with nematode species and strains (Kaya, 1990; Choo *et al*., 2002; Chen *et al.,* 2003). Low temperatures seem to be the main barrier for EPN use in temperate regions (Rutherford *et al*., 1987; Ali *et al.,* 2007; Pervez *et al.,* 2008) and induces inactivity of infective juveniles and is characterised by decreased enzymatic activity and mobility, both reducing metabolic expenditures (Molyneux, 1985; Fan and Hominick, 1991).

Kaya (1977) reported that *S. carpocapsae* did not develop at 10 ˚C and above 33 ˚C, whereas between 15 ˚C and 27 ˚C the IJs developed and reproduced, with the optimum at 25 ˚C. The optimum temperature for activity of *S. carpocapsae* was determined in the temperature range from 22 to 24 ˚C (Choo *et al.,* 2002), *H. bacteriophora* from 22 to 26 ˚C (Doucet *et al.,* 1996), and *S. feltiae* at 25˚C (Belair *et al.,* 2003). However, Blackshaw and Newell (1987) reported that the optimum temperature for *H. heliothidis* to infection and penetration was 28 ˚C, while the range was 12 to 32.3 ˚C. The present findings on the temperature preference of the EPN for infection was vary with the EPN species and strains and Such nematodes are able to survive at habitat temperatures that undergo daily and/or seasonal cycles of fluctuation.

Penetration of the IJs of *Steinernema* spp. and *Heterorhabditids* sp. showed highest penetration and was superior to *Oscheius* spp. The IJs penetration to *C. punctiferalis* larvae was significantly affected by the temperature, never theless the rate of penetration is also depends on the IJs infection strategies (cruiser and abuser). The rate of penetration could be used as a real measure of host infection. These results might suggest that the new isolates were of different species or natural variability within *C. punctiferalis* larvae. However, Tomalak (2004) observed variation in IJs penetration between native isolates of EPNs originating from collection sites located within a short distance, suggesting that variation in infection can be observed between EPNs of different species but also between strains of the same EPN species (Mwaitulo, 2011).

Entomopathogenic nematodes have not previously been used for the biological control of insect pests infesting ginger, and this report indicates their potential as a biological control agent against the key pest of ginger in India. The present study shows that temperature can affect host recognition, penetration, infectivity and multiplication of EPNs. At lower temperature these nematode activities are reduced. Among test EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 03) and *O. gingeri* showed promise at 30 ˚C in laboratory bioassays against *C. punctiferalis*. A temperature of 30 ˚C was also optimum for culturing of EPNs and for maximum numbers of IJs to be used for research purposes or field application.

ACKNOWLEDGEMENTS

The authors express their gratitude to Director, Indian Institute of Spices Research, Kozhikode for providing all the facilities and Mr. K. Jayarajan, Technical officer, IISR, Kozhikode for statistical analysis.

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